I. THE CHANGES IN COMMERCIAL SUGAR SIRUPS DURING MANUFACTURE AND AGING

II. DETERINATION OF VANILLIN AND COUMARIN IN FLAVORING EXTRACTS BY AN ULTRAVIOLET ABSORPTION METHOD

III. THE CAROTENOIDS IN CORN AND THEIR SUSCEPTIBILITY TO BLEACHING

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1944

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Acknowledgment

The author wishes to express his most sincere appreciation to Professor D. T. Englis for his constantly cheerful and helpful interest in the preparation of this thesis.

The author is indebted to the A. E. Staley Manufacturing Company for supplying the commercial corn sirups and the other corn sirups prepared by laboratory methods.

I. THE CHANGES IN COMMERCIAL SUGAR SIRUPS DURING MANUFACTURE AND AGING

SECTION I

Introduction

For many years, color production in sirup has been of great importance to the manufacturers, especially from the sales angle. As is known, the color increases upon aging, especially in the regular untreated commercial corn sirups.

It has been found that the addition of 0.007% SO_2 as sodium bisulfite has a pronounced effect on improving the color stability on aging. This amount is the range used by the industry in domestic sirups, but is not as effective as is desired. Although various oxidizing and reducing agents have been tried at one time or another, none has been found to be as effective as bisulfite.

The amount of literature on this subject is limited and the theories concerning color production are varied. Previous work[1,3] done on this problem in this laboratory indicated that furfural may be one of the impurities causing color formation. Also levulinic acid may be present and in combination with the furfural may contribute to the discoloration.

The present investigation was carried out in an attempt to identity, by means of the ultraviolet absorption spectra, the substance or substances present in corn sirups, which are responsible for the coloration.

Experimental

A Bausch and Lomb Medium Quartz Spectrograph was used for the majority of the ultraviolet absorption determinations. It was supplemented with a Leeds and Northrup recording microphotometer. Later, a Beckman Quartz Spectrophotometer was acquired and used in the last part of the problem.

Samples of corn sirups, prepared according to the usual commercial methods but of different ages, with and without sodium bisulfite as a color inhibitor, were examined. Other samples clarified with decolorizing carbon, were similarly examined. Two other sirups, one of which was made from a starch extracted

with alcohol and the other sirup clarified by a special new process, presumably by an exchanger method, were examined. Some of these samples were subjected to an accelerated aging test, then examined for absorption in the ultraviolet region. After standing for 6 months at room temperature, these sirups were re-examined.

The experiments indicated that furfural or some furfural type of compound is the principal substance which develops during the aging process which shows absorption in the ultraviolet region. The quantity does not appear to increase continuously beyond a moderate concentration level. Apparently the furfural body is only a step in a series of interrelated reactions. The presence of sulfur dioxide lowers the absorption peak of the furfural, presumably by the formation of an addition product to the aldehyde group. As a result, the tendency toward further reaction is inhibited.

Examinations were also made of the absorption of pure furfural and pure levulinic acid, the most probable degradation products of the sugars. These substances were tested alone, in mixtures, and in the presence of sugars, bisulfite, before and after aging tests. When furfural alone was aged, a decrease in absorption in the ultraviolet range took place, showing a transformation to some other compound. In the concentrations studied it was not possible to establish that this compound gave rise to color in the visible range. The sulfite and aging effects in the presence of pure sugars were of the same nature as observed for commercial sirups. In the instances where no change in the amount of furfural was noted it may be postulated that the rates of formation and further transformation of this compound were equal, or that some protective action by the sugars prevailed. Levulinic acid showed only slight absorption in the utraviolet region. The effect of its presence cannot be concluded with certainty.

As representative of the nitrogenous impurities and their probable effects, solutions of monosodium glutamate, furfural-monosodium glutamate-saturated dextrose, both heated and unheated, were examined in the ultraviolet region. Also samples of tryptophan, furfural-tryptophan, and furfural-tryptophan-high purity sirup, which were also accelerated aged, were examined in the ultraviolet region. These systems are very complicated and

the data so far obtained is not adequate to interpret the effect of the nitrogen materials.

Addition of small amounts of copper and iron salts to the highly purified sirup showed little influence upon color formation under the conditions studied.

A solution of an undialyzed caramel product gave an absorption curve similar to that of furfural and of commercial sirups. The effect of sulfite addition was similar. This gives further evidence for the belief that the formation of furfural compounds constitutes the first step in sirup discolorization, and that these substances serve as precursors of the compounds showing absorption in the visible range. Although other factors may be involved in the darkening of sugar products, it is believed that the carmelization process, even though it proceeds very slowly at room temperature, is the principal one responsible for the changes in sirups of high sugar purity.

SECTION II

CHANGES IN AUTOCLAVED DEXTROSE SAMPLES

Introduction

In studying the browning of autoclaved milk, Kass and Palmer[6] upon the basis of experiments in which lactose solution were heated with buffers observed that the discoloration was accompanied by a development of acidity, pronounced fall of optical activity, slight loss of copper-reducing ability, and an appreciable but constant conversion of lactose to ketoses, or substances not oxidized by sodium hypiodite. Since dextrose, perhaps the most reactive constituent in the corn sirups, may be expected to show the same general conduct as lactose it seemed desirable to follow the changes in dextrose after it had been autoclaved in the presence of various substances and in this way obtain an idea of the possible changes in sirups during their preparation.

Experimental

Solutions of 4% Dextrose and 40% Dextrose, containing various constituents, were autoclaved for periods of 30 minutes

and 60 minutes at a pressure of 15 lbs. / sq. in. The samples were examined for total reducing sugars, aldoses, ketoses, optical activity, and color formation.

In the presence of phosphate, there was a conversion of the aldose to ketoses. Calculations based on change in rotation and the quantity of ketoses found by chemical method indicate that although levulose may be the chief ketose formed, some other ketose of dextro, or lesser levo, rotation than levulose must have been produced.

In the absence of phosphate, the conversion of aldose to ketose and the production of color was slight.

A marked yellow to brown color was characteristic of these sugar solutions containing phosphate. When small amounts of ammonium salts were present also, the color was only slightly different. However as the quantity of ammonium salts was increased much greater darkening was noted.

The addition of SO_2 as $NaHSO_3$ to the samples containing phosphate before autoclaving reduced the visible color in the autoclaved samples, but apparently did not affect the amount of ketose formed.

PART II. DETERMINATION OF VANILLIN AND COUMARIN IN FLAVORING EXTRACTS BY AN ULTRAVIOLET ABSORPTION METHOD

Introduction

One of the early methods developed for the determination of vanillin and coumarin in flavoring extracts was that proposed by Hess and Prescott[4], a modification of which is in general use today[8]. This method is based on the differences in the chemical structure of vanillin and coumarin. Vanillin, m-methoxy, p-hydroxy benzaldehyde, is separated from coumarin, an inner lactone of o-hydroxycinnamic acid, through the addition of aqueous ammonia to an ether extract of the vanilla extract. The vanillin forms a water soluble aldehyde-ammonia while the coumarin remains unaffected in the ether layer. Upon acidification and extraction of the aqueous layer with ether, the vanillin is recovered in the ether layer. By evaporating the ether solutions, the

amounts of vanillin and coumarin can be determined. However, the residues are usually impure and need further purification, thus reducing the convenience and the accuracy of the method.

Since the development of the Hess and Prescott procedure, several methods have been proposed for the determination of vanillin alone and coumarin alone in flavoring extracts.

Both vanillin and coumarin show pronounced absorption in the ultraviolet region of the spectrum. Vanillin exhibits a maximum at a wavelength where coumarin shows only slight absorption. It then seemed possible that the principle used by Englis and Skoog[2], for the determination of sulfanilimide and sulfathiazole in a mixture, could be applied in this case.

It was the purpose of this work to develop a method for the determination of vanillin and coumarin in vanilla extracts by means of ultraviolet spectroscopy.

Experimental

Some of the absorption data were obtained by means of a Bausch and Lomb Medium Quarts Spectograph supplemented with a Leeds and Northrup recording microphotometer. Other measurements were obtained with a Beckman photoelectric spectrophotometer.

Blends of Genuine and Imitation Vanilla Extracts—A method was developed for the determination of vanillin and coumarin in blends such that any commercial extract could be analyzed by this procedure.

The procedure involves a dilution, precipitation with lead acetate, extraction with ethyl ether and dilution of the ether extract to a large volume with water. The quantity of total vanillin and coumarin in the solution is estimated from the absohption at 2875A and the quantity of each form a simple equation involving values at 2313Å.

Imitation Vanilla Extracts.—Various clarifying agents, such as norite, filteraid and alumina cream, were used in an attempt to remove the coloring matter in the imitation extracts, but they were found to be unsatisfactory. Consequently, it was decided to use an ether extraction method for the separation of the vanillin and coumarin.

This method was as follows: a small volume (5 ml.) of the vanilla extract was placed in a 10 ml. separatory funnel and extracted with 5 ml. portions of ehtyl ether. After each extraction the aqueous layer was carefully withdrawn into another 10 ml. separatory funnel and the extraction repeated. After four such extractions, the ether extractions were all washed into a 1000 ml. volumetric flask and the mixture diluted to volume. Then the absorption of the solution was determined. Again by use of the same equation as used with the blends, the vanillin and coumarin were calculated.

Pure Vanilla Extracts.—Two procedures for the determination of vanillin in pure vanilla extracts were developed. The first involved the addition of lead acetate to the extract to precipitate the resins, then a dilution to a large volume with water. The sample was filtered and the filtrate treated with $NaH_2PO_4H_2O$ and filtered before the absorption was determined. From the observed E values at wavelength 2313Å the quantity of vanillin in 100 ml. of extract was calculated by reference to the E values for solutions of known concentration at this wavelength. An alternate procedure was suggested wherein the resins were first precipitated with lead acetate, then a portion of the resin-free filtrate extracted with ethyl ether and the ether extract, which is small in volume, is diluted to a large volume with the absorption determined at 2313Å.

The results obtained for the analysis of added constituents agreed within an average of ± 2 mg. for 200 mg. of the substance present. The method is rapid, simple and requires a much smaller volume of sample for analysis than the official method.

PART III. THE CAROTENOIDS IN CORN AND THEIR SUSCEPTIBILITY TO BLEACHING

Introduction

It was noted that one variety of yellow hybrid corn, Pioneer 335, was markedly bleached by the action of sunlight while another variety, Pioneer Hybrid 324, was not noticeably affected. After exposure to rain, the bleaching of the susceptible hybrid by sunlight was even more pronounced while the resistant variety was still unaffected.

The problem of establishing the changes which occur after the sample was exposed to the sunlight and moisture was undertaken by Jensen[5], who found a slight decrease in the pigment content of the hybrid corn after exposure.

Since it has been shown that corn has a Vitamin A value[7], it was thought advisable to investigate, by spectrophotometric methods, the possibility of correlating changes in the pigment of corn as indicated by observation in the visible region with a corresponding change in the amount of Vitamin A or the fraction absorbing in the ultraviolet region.

Experimental

A Beckman Quartz Spectrophotometer was used in all the absorption determinations.

An investigation was carried out in which two samples of corn, Hybrids 335 and 324, were examined for their cryptoxanthin and apparent Vitamin A content. The process of analysis involved saponification, followed by an extraction with petroleum ether, then a spectrophotometric examination of the isolated fraction at 3250Å for apparent Vitamin A and 4500Å for the cryptoxanthin.

Hybrid 335 and Hybrid 324 were exposed to a strong light for two hours and under varying conditions. Hybrid 335 was also exposed to ultraviolet light under similar conditions.

No significant changes in the carotenoid and apparent Vitamin A content of the susceptible and resistant hybrids were found. Exposing a very thin layer to a strong light did not change the results.

It is concluded that any change in the pigment content as a result of the bleaching of the susceptible hybrid is within the experimental error of duplicate analyses and that an apparent bleaching action is a result of a change in the transparency of the pericarp.

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Vita

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